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LETTERS AND CORRESPONDENCE

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Pure Red Cell Aplasia Complicated by Angioimmunoblastic T-Cell Lymphoma: Humoral Factor Plays a Main Role in the Inhibition of Erythropoiesis From CD34$^+$ Progenitor Cells

To the Editor: Pure red cell aplasia (PRCA) has heterogeneous pathogenicity. A variety of hematologic malignancies, especially lymphoproliferative disorders have been reported to be associated with PRCA [1,2]. Angioimmunoblastic T-cell lymphoma (AILD-T), described in REAL classification, is clinically characterized by generalized lymphadenopathy, hepatosplenomegaly, constitutional symptoms and various immunologic disturbances. A few cases of AILD-T with PRCA have been reported [3,4], and the pathogenesis has not been identified. We report a case of AILD-T with PRCA, and demonstrate in vitro inhibition of erythropoiesis from the normal bone marrow progenitor cells by using the patient's serum.

A 46-year-old man with AILD-T was admitted to our hospital because of systemic lymphadenopathy, hepatosplenomegaly, massive pleural effusion, and severe B-symptoms. The diagnosis of AILD-T was established by cervical lymph node biopsy. Hematological findings were as follows: hemoglobin 7.8 g/dl, reticulocytes 0%, platelets 112 × 10^9/l, leukocytes 3.6 × 10^9/l (with normal hemogram). Serum erythropoietin was elevated to 254 mU/ml (normal: 8–36). Bone marrow aspirate and biopsy showed the lymphomatous infiltration and aplasia of erythroid precursors with normal myelopoiesis and megakaryopoiesis. The patient received chemotherapy and achieved remission. After chemotherapy, the hematological and bone marrow findings showed the improvement of PRCA and the serum erythropoietin normalized.

In vitro culture of bone marrow progenitor cells were performed. Normal bone marrow cells were obtained from a healthy donor after obtaining informed consent. CD34$^+$ progenitor cells were purified by immunomagnetic separation (MACS system; Miltenyi Biotec, Bergisch Gladbach, Germany). Colony-forming assay were performed in triplicate using premade methylcellulose medium (Stem Cell CFU Kit; Baxter, Deerfield, IL, USA) which contained 3.0 U/ml of erythropoietin. The patient's serum obtained before and after chemotherapy was added at the final concentration of 10%. Normal AB serum and the serum from another patient with AILD-T without PRCA were used as controls. CD34$^+$ cells were plated at 1 × 10^3/ml and all cultures were maintained in a CO_2 incubator. Burst-forming unit-erythroid (BFU-E) was counted after 14 days of culture. The results are presented in the Table. The patient's serum before treatment significantly inhibited the BFU-E formation compared with controls ($P < 0.05$ by Student's *t*-test). However, the serum obtained after chemotherapy did not decrease BFU-E. No differences were seen in the numbers of colony-forming unit granulocyte-macrophage (CFU-GM) among the serum samples.

In AILD-T, only 1 report was published which analyzed the humoral effect in the clonal study of erythropoiesis [3]. They cultured normal bone marrow cells and demonstrated the inhibition of erythropoiesis by the serum from a patient. Our study, culturing purified CD34$^+$ progenitor cells, showed the significant inhibition of BFU-E by the patient's serum before but not after chemotherapy. The findings suggest the significance of the humoral factor in the pathogenesis of PRCA at the hematopoietic stem cell level. AILD-T is characterized by the immunological disorders and also by the release of several cytokines which often enhance hematopoiesis [5]. Not only immunological mechanisms, but also cytokines may be involved in the pathogenesis of PRCA with AILD-T. Further study focused on the type of humoral factor should be performed.

TABLE I. Colony-Forming Assay From CD34$^+$ Bone Marrow Cells

Serum[a]	BFU-E/10^2 CD34$^+$ cells
Control 1	50.2 ± 4.6
Control 2	48.5 ± 14.1
Before chemotherapy	24.4 ± 6.2[b]
After chemotherapy	50.5 ± 6.5

Values are means ± SD of triplicate cultures.
[a]Control 1; normal AB serum, Control 2; serum from a patient with AILD-T without PRCA.
[b]Significantly decreased compare with controls ($P < 0.05$).

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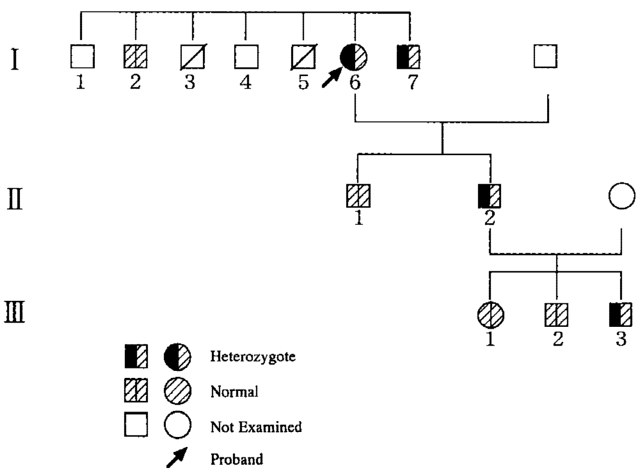

Fig. 1. The pedigree chart.

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A Novel Mutation of the Protein C Gene With a Frameshift Deletion of 3 Base Pair (^{3380}AGG) in Exon 6 in Type 1 Deficiency Associated With Arterial and Venous Thrombosis

To the Editor: Protein C deficiency and other inherited thrombophilic disorders such as deficiency of antithrombin III, protein S, and activated protein C resistance due to factor V Leiden gene mutation, are commonly associated with risk factors of venous thrombosis [1,2]. We report here, a rare case of heterozygous type 1 protein C deficiency with arterial thrombosis, renal and splenic infarctions, and venous thrombosis of pulmonary embolism due to a novel mutation of the protein C gene.

A 62-year-old female was hospitalized in April 1998 for recurrent acute epigastralgia. Gastric endoscopy did not reveal any lesion. Enhanced computed tomography revealed right renal and splenic infarctions. CBC and blood chemistry were almost normal except for mild thrombocytosis, 41.4 $\times 10^9$/L. The coagulation study revealed PT 12.8 sec (control 14 sec), and aPTT 41.4 sec (control 36 sec). Anticardiolipin IgG antibody, lupus anticoagulant, anti-beta 2 glycoprotein 1 antibody, and anti-DNA antibody, were all negative. She had two sons, and no abortion or premature labor.

The pedigree chart is shown in Figure 1. The levels of protein C antigen and activity of the proband (I-6) were reduced to 36% and 39% of normal, respectively. The levels of protein S antigen and activity were both normal. Three other asymptomatic heterozygotes were found.

Molecular analysis of genomic DNA of protein C was performed by direct sequencing in the heterozygotes, the proband, youngest son (II-2), and in normal control, a grandchild (III-1). A novel mutation of frameshift deletions of 3 base pair from the nucleotide position 3380 to 3382 in exon 6, AGG [4], was found in the proband and the youngest son, whereas analysis of the grandchild revealed a normal sequence. The deletion results in the amino acid deletion of Glu 112 in the region of the second epidermal growth factor (EGF)-like domain [5].

Anticoagulant therapy with warfarin was started, and the patient remained asymptomatic for 6 months. However, she complained of sudden right chest pain in November 1998, and pulmonary blood flow scintigraphy revealed a right pulmonary embolism in S9.

The protein C mutation database contains 160 unique mutations in the protein C gene from a total of 315 unrelated probands [6]. The majority of these patients are heterozygous for type 1 deficiency [6]. Most of the mutations are missense mutations, and the remaining are nonsense mutations, promoter mutations, mRNA splicing mutations, insertions, and deletions [6]. A total of 12 short deletions between 1 and 18 base pair were reported [6]. Frameshift deletions were reported in 10 of 334 mutants [6]. The mutation in our case has not been previously reported [6].

Venous thrombosis is typical and deep vein thrombosis of the lower limbs with or without pulmonary embolism accounts for approximately 90% of all thrombotic episodes in heterozygous protein C deficiency [1–3,5,6]. The arterial thrombosis as in our case was rare and accounted for 3% to 7% of thrombotic episodes [2]. The risk factors for venous thrombosis are surgery, pregnancy, and immobilization [1], however, those in arterial thrombosis are less well established, and are considered to be athero-arteriosclerosis, diabetes, increased blood viscosity, paroxysmal nocturnal hemoglobinuria and thrombocytosis [3]. The present case had mild thrombocytosis. The median age at occurrence of arterial thrombosis was 52 years and was higher than in the venous thrombosis of 36 years [2].

It remains unknown how the present mutation of the protein C gene in the region of the second EGF-like domain and the resulting abnormality of the protein C structure are related to type 1 deficiency with such a clinical manifestication. Further study of the present case is in progress.

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Unique Sequence of Pernicious Anemia, Stomach Cancer, and Myelodysplastic Syndrome

To the Editor: We described here a case of myelodysplastic syndrome (MDS) subsequent to pernicious anemia (PA) and stomach cancer (SC).

A 76-year-old man was admitted to the hospital in 1994 and hematological examinations revealed macrocytic anemia with a red blood cell count of 1.32×10^{12}/l, hemoglobin concentration of 6.0 g/dl, hematocrit of 17%, reticulocyte count of 7.92×10^9/l, leukopenia with leukocyte count of 3.1×10^9/l, and thrombocytopenia with platelet count of 74×10^9/l. Peripheral blood film showed hypersegmentation of neutrophils, and bone marrow examination revealed megaloblastic erythropoiesis and normal chromosomal karyotype. The serum cobalamin level was low (30 pg/ml, normal 249–938 pg/ml). Anti-intrinsic factor (anti-IF) antibody was present. The urinary methylmalonic acid excretion increased. Findings of other immune examinations (Table I) were negative, except for slight increases of serum immunoglobulin (Ig)G and IgA. A diagnosis of PA was established and the patient was treated with cobalamin. All hematological data were within normal range 3 months after the treatment began. SC was detected at this time. Hematological findings were stable after surgery without chemotherapy for the cancer until December 1998, when laboratory examinations disclosed pancytopenia with a red blood cell count of 1.29×10^{12}/l, hemoglobin concentration of 4.5 g/dl, hematocrit of 14.4%, reticulocyte count of 23.2×10^9/l, leukopenia with leukocyte count of 2.3×10^9/l, and thrombocytopenia with platelet count of 79×10^9/l. Peripheral blood film showed degranulated neutrophils associated with Pelger-like nuclei, circulating micromegakaryocytes, and blasts (1% of white blood cells). The bone marrow was hypercellular and showed megaloblastoid changes, neutrophilic immaturity, and micromegakaryocytes. Cytogenetic analysis of bone marrow cells revealed trisomic 47 XY,+8. Serum level of cobalamin was normal. Immune examinations showed positive results for anti-IF antibody, antinuclear factor, immune complex, rheumatoid factor, and increased serum IgG and IgA as shown in Table I. A diagnosis of refractory anemia with excess myeloblasts was made and the patient was treated with blood transfusions because he was elderly and only complained of anemic symptoms.

Several studies have suggested an association between PA and several autoimmune diseases. MDS is a clonal hematopoietic disorder and its possible association with immunological abnormalities [1]. Clustering of autoimmune diseases in individuals is clearly recognized and certain disease associations have been established. Immunological features between PA and MDS in our case were different as shown in Table I, although this case highlights the immunogenetic links between the disorders with immunological aberrations and draws PA and MDS further into the spectrum.

An increased risk of SC has been demonstrated and hematologic malignancies have been repeatedly observed in case reports, including myeloid leukemia [2], polycythemia vera, and multiple myeloma following the diagnosis of PA. Brinton et al. [3] and Hsing et al. [4] performed large-scale, population-based, prospective studies that showed a significantly higher incidence of myeloid leukemia following a diagnosis of PA. The incidence of preleukemia or MDS has not been evaluated. Mufti et al. [5] observed three cases of MDS associated with PA, although hematological and clinical features were not sufficiently shown. The association between PA and MDS in case reports may be accidental, although large-scale studies on the occurrence of MDS subsequent to PA are needed to clarify the relationship between PA and MDS with immunological abnormalities

TABLE I. Immunological and Cytogenetic Studies

	Feb. 1994 (Diagnosis: PA[a])	Dec. 1998 (Diagnosis: MDS[b])
Anti-IF[c] antibody	+	+
Anti-PC[d] antibody	−	−
Thyroid test	−	−
Microsome test	−	−
Anti-thyroglobulin antibody	−	−
Anti-TPO[e] antibody	−	−
Anti-nuclear factor (<×40)[f]	<×40	×160
LE[g] test	−	−
Anti-DNA (<6 IU/ml) antibody	<6	<6
Anti-RNP antibody	−	−
Anti-Sm antibody	−	−
C3c(63–134 mg/dl)	42	96
C4 (13–36 mg/dl)	21	25
CH50(30–40 CH50 U/ml)	30.9	39.1
Immune complex (C1Q) (<3 μg/ml)	<1.5	4.8
RA	−	+
RAPA (<×40)	nd	×1280
IgG(870–1700 mg/dl)	2013	3289
IgA(110–410 mg/dl)	473	711
IgM(33–190 mg/dl)	116	110
Chromosome	46,XY[100%]	46,XY [80%] 47,XY,+8[20%]

[a]PA, pernicious anemia;
[b]MDS, myelodysplastic syndrome;
[c]IF, intrinsic factor;
[d]PC, parietal cell;
[e]TPO, thyroid peroxidase;
[f]( ), normal range;
[g]LE, lupus erythematosus.

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Long-Term Response in Accelerated-Phase Chronic Myelogenous Leukemia With Protracted Splenic Irradiation and α-Interferon

To the Editor: Chronic myelogenous leukemia (CML) in accelerated phase portends an ominous outcome. No optimal treatment is available and most patients will succumb to their disease within the year following the diagnosis [1,2].

A 34-year-old man diagnosed as having CML in chronic phase was referred to us in October 1989. His white blood cell count (WBC) was of 76×10^9/l with medullary and circulating positive Ph+ cells. Because no HLA-identical sibling donors were found, the patient was treated orally with hydroxyurea (HU). Five years later, despite the addition of ARA-C and IFN-α, the disease entered in accelerated phase with WBC of 126×10^9/l with 12% basophils and 13% blast cells associated to a painful massive splenomegaly. Palliative radiotherapy to the spleen was started in December 1996. WBC plummeted to 2.4×10^9/l with no circulating basophils or blasts after a radiation dose of 3 Gy. Two months later, protracted splenic irradiation (0.5–1 Gy per week) was resumed because of a significant increase in WBC counts and spleen size. Eight months later, INF-α was introduced in combination with radiotherapy. After 6 months of combined therapy, bone marrow aspiration showed a persistent chronic phase with 100% positive Ph+ cells. Radiotherapy (total dose of 49.5 Gy) was discontinued, and chemotherapy combining INF-α and low-dose HU was initiated. A year later, CML was still in chronic phase with the patient having resumed full-time work. According to the multivariate analysis recently published by Rodriguez et al. [3], our patient presented multiple prognostic factors associated with a shorter survival. Time from diagnosis had exceeded 7 years, the spleen was larger than 10 cm, PB basophils were >7%, and PB blasts were >3%. Treatment options in CML in accelerated phase are very disappointing. Bone marrow transplantation (BMT) yields durable remissions in a small percentage of patients [4,5], conventional chemotherapy induces transient responses but has no impact on overall survival, and radiotherapy is always used with a palliative intent. In our study the main important conclusion was that moderate doses of radiotherapy (3 Gy) followed by protracted radiation delivering small weekly doses to the spleen were capable of reversing CML from an accelerated phase to a chronic phase.

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